A CONTRIBUTION TO THE LIFE HISTORY OF *AMŒBA PROTEUS* LEIDY.[1]

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It is only within the last few years that a connected life history of *Amœba proteus* has begun to be formulated. This does not seem strange, even in view of its common use in laboratories, since the difficulties attendant upon the study of the life cycle of such an elusive form, are numerous.

The isolated observations upon the various activities of *Amœba*, the accounts of suppositious appendages, and the descriptions of supposedly new species or varieties, are numerous. The fact that so much observational material upon so many apparently nearly related forms has accumulated, and that so many diverse accounts of the habits of one and the same form have been given, has led some investigators to suspect that perhaps much of this material might be combined to throw light upon the life history of possibly some one or two valid species in whose life cycles some of the forms heretofore described might prove to be merely developmental stages. This suspicion has been strengthened recently by the fact that some of the *Rhizopoda* closely allied to the *Amœba proteus*, whose life cycles are now known, show both widely varying forms and habits during different stages in their growth and reproduction.

Investigations of the life history of *Amœba proteus* which have hitherto been made make it possible to generalize, to some extent, upon the course of some of the changes which take place during its development. There seem to be several modes of reproduction in this form, and several intermediate stages between young and adult before the life cycle is completed. In this communication an attempt is made; first, to review briefly what we know of the life history of *Amœba proteus;* second, to

[1] Grateful acknowledgment is here made of the aid of Professor H. D. Reed, of the department of zoölogy, Cornell University.

describe an apparently hitherto unknown method of reproduction; and third to record some observations upon the development and growth of the forms produced by this new method.

The first observer to suggest that another method of reproduction other than simple fission might obtain among the *Amœba* was Varter ('56) who recorded the presence of numerous fine granules which he observed filling the bodies of some *Amœba radiosa*. These granules he looked upon as fragments of the nucleus, and since, when they escaped from the body, they moved with a curious jerky motion, he inferred that they must be provided with flagella, and termed them spermatozoids. The observations extended no farther. Again in 1863 he observed the granulation of the nucleus in *Amœba princeps*, noting that the original nucleus divided several times and gave rise to many smaller nuclei, which he termed, though apparently with meager foundation, reproductive cells.

Wallich ('63) noted that when *Amœba villosa* "died," the nucleus, which had divided several times, escaped from the body surrounded by bits of protoplasm, of globular form. He observes that the fate of these globules is unknown to him, but believing that they might function in some reproductive process, termed them scaroblasts. Referring to the many different species of *Amœba* which appeared in his cultures, he writes (with reference particularly to *Amœba princeps*, *diffluens*, and *radiosa*): "It will, I think, eventually be found that these are mere transitory phases of one and the same species." Later, he observes with greater conviction: ". . . though not prepared to affirm that the whole of the varieties of *Amœbæ* are reducible to a single, primary, specific type, I candidly confess that the balance of the evidence appears to me to point to such a conclusion, and to indicate that the divergence in the form and outward characters may be wholly dependent on the local and even temporary conditions of the medium in which the young animal happens to make its appearance in the world."

It is to Scheel ('99) that we owe our first complete knowledge of a type of reproduction different from the familiar method of binary fission. In 1899 he described a process of reproduction in *Amœba proteus* which he termed *schizogony*, after a somewhat

similar mode of multiplication occurring among the *Sporozoa*. As it occurs in *Amœba proteus* its course is as follows: The individual encysts; the nucleus divides into several smaller nuclei; these migrate to the periphery of the cell; the protoplasm of the cell divides into as many equal portions as there are nuclei; the cyst wall ruptures; and the nucleus-containing bits of protoplasm emerge, each a complete *Amœba* and an epitome of its parent. In analogy with the spores of the *Sporozoa* these were given the name, pseudopodiospores. More recently Calkins ('05) has noted a method of reproduction in which the nucleus produced by repeated division, gametic nuclei, whose fusion resulted in other nuclei which became the nuclei of the smaller individuals after the manner of the pseudopodiospores just mentioned. The young *Amœbæ* soon assumed the form hitherto called *Amœba radiosa;* passed through this stage and became the common *Amœba proteus*.

Another apparent mode of reproduction was observed by Metcalf ('10). In this method the observer is of the opinion that the sequence of events is: Mature individuals produce globular masses of protoplasm, which are termed gemmules. These develop flagella and assume a cercomonadoid form; later they fuse by two and two, lose the flagella, and develop into adult *Amœbæ*. It is stated that possibly the life cycle of *Amœba proteus* may require a year or more for its completion, and may exhibit during its course three, or even more, modes of reproduction.

Schepotieff ('08, '09) states: "All of these examples suggest that in the case of the *Amœba* [*proteus*] the developmental cycle may be completed in very different ways."

It has been shown, as we have said, that *Amœba radiosa*, formerly regarded as a distinct species, is merely a stage in the development of *Amœba proteus*. It may be that this also is true of such recognized species of *Amœba* as *villosa*, *princeps*, *diffluens*, etc. It is suggested elsewhere in this paper that the species known as *Amœba guttula* is but a developmental stage in the cycle of *proteus*. Vahlkampf ('05) has shown that *Amœba limax*, at least, is to be regarded as a distinct species.

Preparation of Cultures.

The material from which the *Amœbæ* herein described were reared was obtained from a pool in a cattail marsh, in about two feet of water among decaying lily pads and *ceratophyllum*. The thin glutinous deposit upon the bottom and especially on the lily pads which had fallen to the bottom was found to be rich in *Amœba proteus*. In the laboratory the material was distributed into several battery jar aquaria after having been filtered through cheese cloth to remove the larger creatures.

An immediate examination of the material showed that beside the active *proteus*, *radiosa*, *guttula*, and *limax*, there were many encysted protozoa. One encysted form which appeared in large numbers, and which confusingly resembles some of the smaller encysted *Amœbæ* was *Vorticella microstoma* (Fig. 4). Under ordinary magnification its distinguishing feature, the crescentric nucleus, is not visible. With the 1.8 mm. objective it becomes faintly discernible, but it is best seen, and the cyst is indubitably identified apparently only when stained. Methyl green and iodine gave the best results.[1]

After some weeks had elapsed *proteus* and *radiosa* appeared in large numbers. The material was now transferred to a dozen small petri dishes and kept in a constant temperature of about 75 degrees Fahr. After a space of a fortnight there was begun the transfer of inoculation of *Amœba proteus* to 4 cm. stender dishes, furnished with straw infusion or oak leaf infusion, and free from all protozoan forms. The infusions were prepared by boiling the straw or leaves for several hours, and decanting off the dark brown liquor, to be diluted to the optimum strength. A slimy scum formed upon the surface of the infusions after a few days time, which when stirred up and caused to sink to the bottom furnished a nutritive substance upon which the *Amœbæ* throve.

From the stender dishes individual *Amœbæ* were removed from time to time and kept in shallow cells sunk in slides of unusual thickness. The slides employed were furnished with a device

[1] Methyl green stain: Saturated alcoholic solution methyl green, 3 parts; 2 per cent. aqueous solution acetic acid, 1 part; water 3 parts. Iodine stain: saturated alcoholic solution iodine, 1 part; water, 2 parts. For various protozoan stains, see Hausman, "Fresh Water and Marine Gymnostominan Infusoria" (in press).

for supplying water to take the place of that carried off by evaporation (Fig. 3).

The method of removing individual *Amœbæ* from stender dishes was as follows, and can be used with success for the isolation of any of the larger protozoan forms: A drop of water containing the *Amœbæ* was placed upon an ordinary slide, and, uncovered, searched with the 16 mm. objective and 8 × eye-piece. When *Amœbæ* were located they were removed by means of what is termed an isolation pipette (Fig. 2). A long rubber tube attached at one end to a glass tube drawn out to a very fine tip, and at the other to a small compression bulb, enables one to select and withdraw very minute objects with considerable precision. Both the stender dishes and growing slides were kept at a temperature of 80 degrees Fahr. in a large aquarium jar heated by a carbon filament lamp placed in the bottom, the current being controlled by a rheostat. By means of this simple device unvarying temperatures could be maintained for any desired length of time. The advantage of such a culture oven is that light is admitted freely on all sides (Fig. 1).

Formation of Apseudopodiospores.

One of the stender dishes which had been inoculated with adult *Amœba proteus* proved very productive, the individuals increasing rapidly, apparently by means of binary fission, since many were observed in process of division. The bodies of the largest individuals became filled with minute bodies, which upon staining seemed to be nuclei. The individuals bearing these were extremely sluggish. The pseudopodia were short; exhibited very little movement, changed their shape but slowly, and upon the functional posterior of the body absent altogether (Fig. 5).

After an interval of four days had elapsed a reexamination of some of the sediment from the same stender dish was made and it was found that the numbers of the large *proteus* had appreciably decreased and that their places were taken by a multitude of very small amœboid forms, of an average diameter of 4 or 5 microns. The majority of these exhibited feeble movements of an amœboid kind. Some were globular, some possessed an irregular body outline, though definite pseudopodia were lacking

(Fig. 10). These, I was later led to believe, originated from the bodies of the larger multinucleated *proteus*. Several of these latter individuals while under observation were observed to give rise to smaller individuals of the form already described. The process was as follows: the animal, which had been moving slowly and apparently without much vigor, gradually came to rest with the pseudopodia upon the then functional anterior portion of the body lobate with slightly pointed tips (Fig. 6). The posterior portion became semi-globular and towards this the greater number of the minute suppositive nuclei within the body plasm migrated. After an interval of about seven to ten minutes the ectoplasm surrounding this globular posterior extremity appeared to disintegrate and from the interior there floated forth several hyaline globules about 4 or 5 microns in diameter. These were followed, after a few minutes by several others, and then a constant outflow began that continued until upwards of thirty of the hyaline spheroids had been extruded (Fig. 7). These were apparently identical with the forms which had made their appearance in the culture. Since these are judged not to be fundamentally different from the pseudopodiospores, of Scheel, but since they exhibited no lobose pseudopodia, they are here called for convenience, *apseudopodiospores*. Some of these, immediately after extrusion began to move slowly, bulging the very thin ectoplasm at several points, yet without forming any definite pseudopodia. Others, synchronous with their emergence, disintegrated. Still others floated away without exhibiting any signs of motion (Fig. 8). It may be that these were gametic forms, and fused before further development.

Towards the time of the completion of apseudopodiospore ejection, the parent *Amœbæ* usually gave signs of renewed activity, elongated perceptibly, and then began apparently to make efforts to move away (Fig. 9). However, after an interval of from twenty minutes to half an hour, they disintegrated. Some disintegrated at once, leaving the apseudopodiospores behind, but usually they were extruded from the globular posterior portion of the animal, the anterior part retaining its integrity. It appeared as though not all of the minute nuclei were used up at the time of the production of one "litter" of apseudopodio-

spores, and that some of them (those in the anterior portion) were lost.

M. Popoff ('11) has recorded a type of reproduction in *Amœba minuta*, a marine species, similar to the one described above, with the difference, however, that the resulting spores were gametes, which later fused. Schmidt ('13) likewise, described this same sort of reproductive activity as occurring in another marine species, *Amœba aquitalis*.

The apseudopodiospores however, at least the majority whose development was watched, were not gametes.

The smallest of the young *Amœbæ* (as we shall call them) those which have just been separated from the parent body are about 3 to 5 microns in diameter and are extremely sluggish. The body is sub-globular and changes its outline but little during the very slow movement. No pseudopodia are developed. The protoplasm is clear, and contains a few small, angular, transparent granules. No contractile vacuole could be seen, nor where the creatures observed to feed (Fig. 10).

With growth comes an increase in activity and a progressively greater irregularity of the body outline (Fig. 11), until at length true lobate pseudopodia make their appearance (Fig. 12). The number of granules within the body increases, food is taken by engulfing, and the protoplasm assumes a grayish hue. This color may be due both to the number of particles within the endoplasm and to the augmentation of its volume.

At the time of the appearance of the true pseudopodia the body is unsymmetrical, but as growth proceeds are more or less radiate arrangement of the pseudopodia takes place, at first not well defined, but becoming more and more pronounced with the increase in size (Fig. 13).

The pseudopodia now become more extended, and tend to develop more acuminate tips. With increasing length and sharpness the pseudopods seem to become more rigid, and spine like, and the granules migrate from them into the more globular central mass of the body, leaving them clear (Fig. 14).

During the time when the young *Amœbæ* are passing from the apseudopodia stage to the *radiosa* stage they confusingly resemble, if indeed they are not exactly similar to the species

known as *Amœba guttula* (Fig. 11), and it is suggested that possibly this creature hitherto accorded specific rank, may be merely a developmental stage in the cycle of *proteus*. That *Amœba radiosa* was named from the *radiosa* stage of *Amœba proteus* has been indicated. The *radiosa* stage which we have observed in this developmental series may be similar.

A new type of modification now takes place, as has been said, when the pseudopodia become longer and more spinous. During this stage, in respect to size, configuration, and characteristic spineous, immobile, hyaline, ray-like pseudopodia, the creatures are apparently indistinguishable from the species known as *Dactylosphærium radiosum*. Hence we shall term this stage the *Dactylosphærium* stage (Fig. 15).

The genus *Dactylosphærium* was established by Hertwig, and Lesser ('74) to receive the organism which they described as *Dactylosphærium vitreum*. The species now known as *Dactylosphærium radiosum*, however, was not referred originally to that genus, but to the genus *Amœba*, as *Amœba radiosa*, by Ehrenberg ('30). It was transferred to the genus *Dactylosphærium* by Bütschli ('80), who however erroneously called it *Dactylosphæria*. Cash ('05) says of it: "The body consists of granular protoplasm and when all the pseudopodia are withdrawn it may become spherical or bluntly lobed; or it may assume an active amœboid phase, when it is hardly, *if at all*,[1] to be distinguished from the smaller forms of *Amœba proteus*." It occurs in marshes and pools, "less common than *Amœba proteus, with which it is usually associated*." [2]

It was observed that not all of the young *Amœbæ* acquired this *Dactylosphærium*-like form. Some became small *radiosa*, passed on to large *radiosa*, and thence on to the *proteus* form. It seemed to be the usual thing, however, for the majority to assume the form of *Dactylosphærium*, and the suggestion is made that perhaps *Dactylosphærium radiosum*, like other former species, may be forced to relinquish its specific distinction.

From the bodies of those individuals which had assumed the *Dactylosphærium* form, lobate pseudopodia were occasionally protruded, and at length the creature became almost globular, and

[2] The italics are the author's.

then proceeded to take on a form similar to the adult *proteus*. Such individuals were isolated, and soon grew in size to adult form, and were, apparently, *Amœba proteus*. Fission was observed to take place among some of these, but no further reproductive activities were noted. The entire sequence of events which have been enumerated took place within three months, the cultures being kept in the glass oven already described, at an average temperature of 80 degrees Fahr.

Some of the individuals of nearly adult proportions developed long, whip-like, and flexible pseudopodia, often more like long threads (Fig. 16). These were clear, even the stouter ones being devoid of granules. Gruber ('11) reported a similar type of pseudopodium (there was but one in this case) which was sent out from the body and moved about as if an organ of exploration.

Some of the most bizarre forms occur during the transition period between the smallest form and the *Dactylosphærium* stage. Fig. 14 represents forms, all but one of which were taken from the same slide.

In Fig. 18 an attempt has been made to group the various forms observed in a tentative cycle of development. The nine arbitrary stages appear to occur in the following order:

1. Adult stage.
2. Division of the nucleus, and migration of the nuclei to the posterior extremity of the body.
3. Escape of apseudopodiospores.
4. *Amœba guttula* stage.
5. Small *Amœba proteus* stage.
6. *Amœba radiosa* stage.
7. *Dactylosphærium radiosum* stage.
8. Resumption of amœboid form.
9. Growth to adult size.

BIBLIOGRAPHY.

Bütschli, O.

'80 *Protozoa*, in Bronn, H. G. Klassen und Ordnungen des Thier Reichs, parts 8 and 9.

Calkins, G. N.

'05 Evidences of a Sexual Cycle in the Life History of *Amœba proteus*. Archiv für Protistenkunde, Vol. 5, p. 1.

'07 Fertilization of *Amœba proteus*. Biol. Bull., Woods Hole, Mass., Vol. 13, p. 219.

Carter, H. J.

'56 Notes on the Freshwater *Infusoria* of the Island of Bombay. Ann. & Mag. of Nat. Hist., Vol. 18, p. 115.

'63 On *Amœba princeps* and Its Reproductive Cells. Ann. & Mag. of Nat Hist., Vol. 12, p. 30.

Cash, J.

'05 British *Rhizopoda* and *Heliozoa*. London.

Ehrenberg, H.

'30 Beiträge zur Kenntniss der Organization der Infusorien, etc. Abhl. der Konigl. Akad. der Wiss. Berlin, p. 30.

Grüber.

'11 Ueber Einartige Körperformen von *Amœba proteus*. Archiv. für Protistenkunde, Vol. 23, p. 253.

Hertwig & Lesser.

'74 Ueber Rhizopoden Denselben Nahstehende Organismen. Archiv. für Mikr. Anat., Vol. 10, suppl., p. 35.

Metcalf, M.

'10 Studies upon *Amœba*. Jour. Ex. Zoöl., Vol. 9, p. 311.

Penard.

'02 Faune Rhizopodique du Bassin du Leman, Geneva.

Popoff, M.

'11 Ueber der Entwicklungscyclus von *Amœba minuta*. Archiv für Protistenkunde, Vol. 22, p. 179.

Schaudinn, F.

'03 Untersuchungen ueber die Fortpflanzen einiger Rhizopoden. Arbeit aus dem Kaiserl. Gesundheitsamte, Vol. 19, p. 547.

Scheel, C.

'99 Beiträge zur Fortpflanzung der Amœben. Festschrift zur Siebensigsten Geburtstag von Carl von Kupffer, Jena, p. 569.

Schepotieff, A.

'09–'10 Amœbenstudien. Zool. Jährbucher, Abt. für Anat., Vol. 29, p. 485.

Schmidt, H.

'13 Faunistische und Entwicklungsgeschichtliche Studien an Sarcodinen der Umgegend von Bonn. Archiv für Protistenkunde, Vol. 29, p. 203.

Wallich, C. G.

'63 (*a*) On an Undescribed Indigenous Form of *Amœba*. Ann. & Mag. of Nat. Hist., Vol. 11, p. 287.

(*b*) Further Observations on *A. villosa* and other Indigenous Rhizopods. Ann. & Mag. of Nat. Hist., Vol. 11, p. 434.

Vahlkampf, E.

'05 Beiträge zur biologie und Entwicklungsgeschichte von *Amœba limax*. Archiv für Protistenkunde, Vol. 5, p. 167.

Vejdovsky, F.

'80 Ueber die Rhizopoden der Brunnewässer Prags. Sitsber. der böhm. Gesell. der Wiss. Prag., p. 136.

Plate I.

FIG. 1. Aquarium jar culture oven. *a*, thermometer; *b*, cardboard cover; *c*, aquarium jar; *d*, cultures; *e*, iron tripod; *f*, lamp; *g*, copper wire rack for lamp.

FIG. 2. Isolation pipette.

FIG. 3. Growing slide. *a*, sponge, for absorbing excess water; *b*, cover glass; *c*, glass tube carrying thread, as siphon; *d*, lower half of glass vial, cemented to the slide with balsam, as reservoir.

FIG. 4. *Vorticella microstoma*, encycted.

FIG. 5. Unusually large *Amœba proteus*, with body filled with minute nuclei.

FIG. 6. *Proteus*, resting before extrusion of the apseudopodiospores.

FIG. 7. Extrusion of the apseudopodiospores.

FIG. 8. Immobile, globular apseudopodiospores.

FIG. 9. *Proteus* after extrusion of the apseudopodiospores.

FIG. 10. Active apseudopodiospores.

FIG. 11. Apseudopodiospores during the stage when they resemble *Amœba guttula*.

FIG. 12. Small *proteus* stage.

FIG. 13. *Amœba radiosa* stage.

FIG. 14. Apparent transitional forms between the *radiosa* and the *Dactylosphærium* stages.

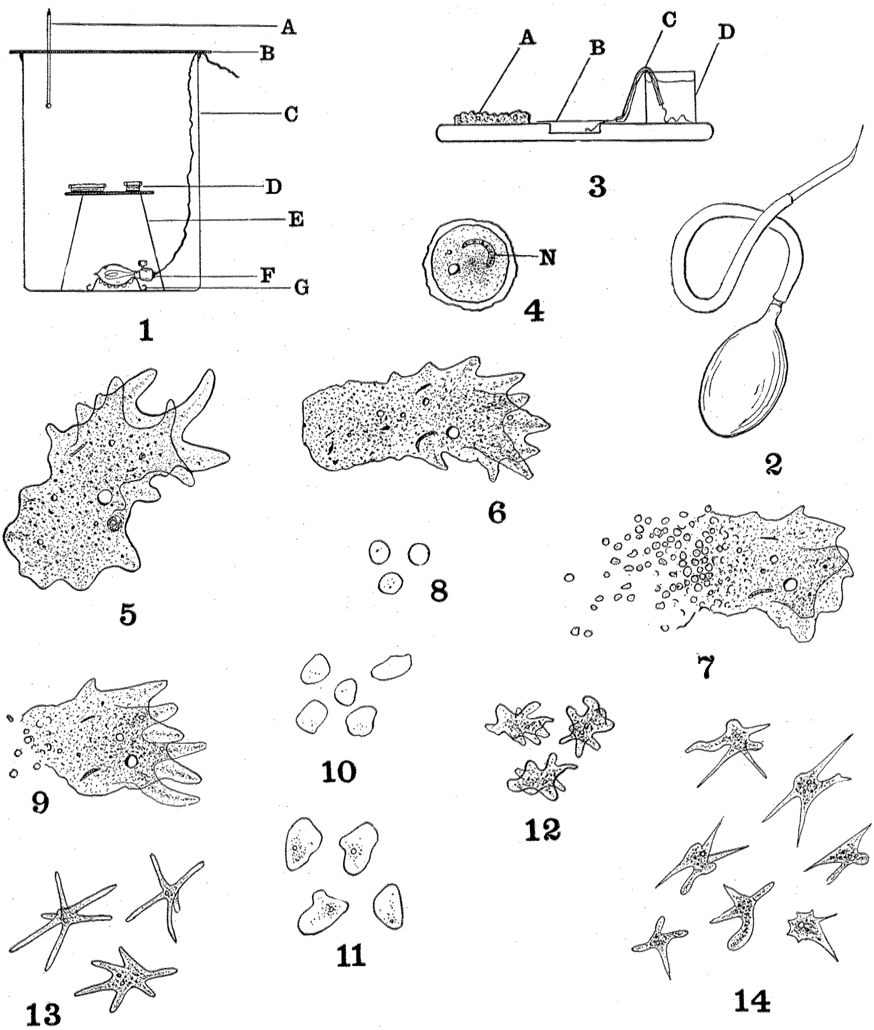
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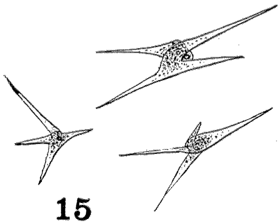
PLATE II.

FIG. 15. *Dactylosphærium radiosum* stage.

FIG. 16. *Amœba proteus* in the adult stage, exhibiting long attenuated pseudopodia.

FIG. 17. Adult normal *Amœba proteus* (stained).

FIG. 18. Tentative Cycle of Development. 1. Adult stage. 2. Division of nucleus; migration of nuclei to posterior. 3. Extrusion of the apseudopodiospores. 4. *Amœba guttula* stage. 5. Small *Amœba proteus* stage. 6. *Amœba radiosa* stage. 7. *Dactylosphærium radiosum* stage. 8. Resumption of amœboid form; retraction of pseudopodia. 9. Growth to adult.



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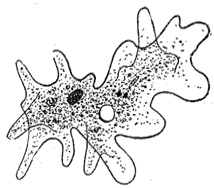

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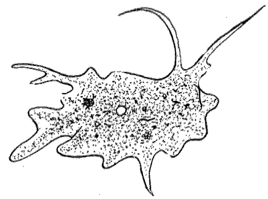

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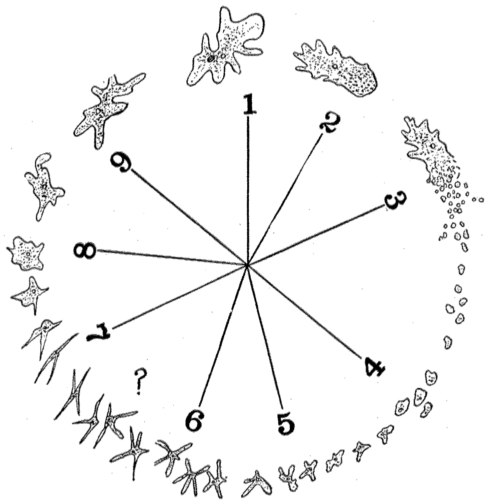

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